



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

have yet found in individual varieties of bats. That of the foot and of the ear, each 4 mm., is great for so short organs. The difference in tragus, 3.5 mm., particularly strikes the attention. I am inclined to believe that this is the least variable organ in bats.

Notwithstanding these differences the closeness of these two bats is very marked, and I should not be surprised if *minor* should eventually prove to be but a variety of *senex*. However, until there is positive evidence that such is the case, it is advisable to consider it as a separate species.

The collector failed to note the date of capture, but informs me that he is certain that it was in July or August, and probably in the former month. From this I imagine that the young bat would have been born some time in September. The specimen was taken at night while flying about a bonfire.—HENRY L. WARD, *Tacubaya, D. F., Mexico, April 20th, 1891.*

EMBRYOLOGY.¹

Some Notes on the Breeding Habits and Embryology of Frogs.—The following notes are the outcome of several years of observations on the breeding habits and stages in the development of frogs. They are confessedly very incomplete, having been collected rather as an amusement than with any desire to increase our present knowledge of amphibian embryology. Some older observations have been verified, and I believe a few new observations made which perhaps are worth recording. From many points of view I think the development of the frog is better adapted to the need of students beginning the study of embryology than the classical chick. Certainly this seems to be true if a clearer knowledge of the phenomena of development in general is desired, and not merely an introduction to human embryology,—the best excuse offered for presenting the hen's egg and chick, with its mystifying yolk and white and its incomprehensible (to the beginner) larval membranes. On the other hand, the ease with which the young chicks are to be obtained at all seasons makes a very strong argument in their favor. Correspondingly, the difficulties of removing the younger stages of the frog's egg from the surrounding jelly has been a great drawback to its study. Appreciating this last difficulty, I have experimented for several years on methods of removing these

¹ Edited by Dr. T. H. Morgan, Johns Hopkins University, Baltimore, Md.

jelly membranes. At last I think that I have successfully solved the problem, and can now obtain with the greatest ease the most difficult stages, which are also in perfect histological condition. The method will be given in the last section—9. Clay models of the early stages of segmentation of the egg have been a very useful addition in presenting to others the arrangement of the cells. These, of course, should be copied from actual eggs, and not from the perfectly regular (but entirely schematic) figures of the ordinary text-books.

The following topics are touched upon: (1) Time of Laying, and Localities. (2) Laying in Confinement. (3) Polar Bodies. (4) Segmentation of the Eggs. (5) Orientation of the Egg. (6) Enclosure of the Light Pole by the Dark Pole. (7) Effect of Temperature. (8) Times of Hatching, etc. (9) Methods of Technique.

1. *Time of Laying, and Localities.*—The observations were made in the vicinity of Baltimore, Md., during the spring months of the years '88, '89, '90, '91. I shall only speak of those species of whose identity I am certain. Other and more imperfect observations are left out. The first frogs to lay, and amongst the very first (*Acris gryllus* excepted) to appear, are the wood frogs (*Rana sylvatica*). A few warm days in early spring suffice to bring them out. The following records give a general idea as to the time: February 23d, '91, and March 8th, 9th, and 10th, '80. The eggs of these had been laid several days. The egg-bunches are found in small pools on the edges of woods, generally amongst the low hills, and are often stuck to twigs of bushes. The bunches are generally large, four to six inches in diameter, and contain very many good-sized eggs. In the same pools it is quite usual to find the firmer egg-bunches of *Amblystoma*, as this Urodele also lays its eggs very early.

Somewhat later two species of tree frogs appear in the small pools in the woods, generally in quite small, and therefore, during the daytime, often quite warm, puddles; sometimes in the same pools as the wood frogs, oftener in the ditches by the side of the road. These tree frogs are *Hyla pickeringii* and *Chorophilus triseriatus*. They are often found paired, and may be in this condition carried to the laboratory, where they continue to lay for hours without abatement. The eggs of these species are very similar, and I know no certain method of distinguishing the one from the other. The bunches are small, attached to bits of grass, or lie simply on the bottom, and each bunch contains from five or six to fifteen or twenty eggs. I have the following records of times at which the eggs were found: *Hyla*—March 9th, 10th, 13th, April 5th, '90; *Chorophilus*—February 23d, '91, March 13th and 24th, '90.

The eggs of *Rana halecina* are found still later, sometimes in the same localities as the wood frogs, oftener in pools in the open ground quite away from the woods. The eggs are individually smaller, so that although the jelly masses are often as large as those of the wood frogs, the number of eggs is greater. The following are the records: March 25th, April 5th, '90. The eggs of *Rana clamitans* are not so certainly referred to its adult, and I have only strong probability showing them to belong to that species. The bunches much resemble those of *R. halecina*, but the eggs are larger and the jelly firmer. Those I have found were also attached to twigs of bushes, which is not always the case in *R. halecina*.

The toad (*Bufo lentiginosus*) in this latitude lays very late in the spring. The eggs are easily distinguished from the frog's, as they are laid in long strings, often yards in length, the eggs arranged (generally) in a single row. They were recorded April 14th, '90, April 5th and 6th, '91. The best localities seem to be those parts of rivers or streams where the water backs up, and to one side protected by a bar, so that the eggs are not carried away by the water, and where the water itself is often exceedingly warm. Copulating individuals are easily obtained, and they continue to lay in confinement.

2. *Laying in Confinement*.—If frogs are caught at the height of the breeding season, they can often be got to lay in confinement. The surest way is to get the paired individuals, frightening them as little as possible, and placing them in dishes or aquaria containing the requisite amount of water. Only once have I had the wood frogs lay in the laboratory, although with proper precaution there seem to be on very great difficulties of obtaining in this way the eggs of this species. A single large bunch of eggs were laid by this pair during the night, which developed normally.

By far the best and easiest eggs to be obtained by bringing frogs into the laboratory are those of the tree frogs named above. They will continue to lay small bunches of eggs for as much as twenty-four hours after catching them. By removing the bunches as fast as laid, an exact record may be kept as to the age of the different lots. Moreover, the eggs of these species are small and the jelly clear, so that they are well adapted for study of the segmentation stages under the microscope. The distinction between the cells derived from the black (animal) pole and those from the yellow (vegetative) pole is very sharp, and the fate of the cells more easily traced through the later stages of segmentation. Toads brought into the laboratory and placed under proper conditions continue to lay for many hours. A single

copulating pair, which were laying eggs when captured, were isolated *over night* from other individuals, and in the morning a long string of eggs were found. Dr. E. A. Andrews carefully estimated the number of these, and found that inside of ten hours the female had laid the astonishing number of 28,000 eggs, and the male had fertilized them. This was at the rate of forty-one eggs per minute for ten hours. After the eggs are laid the male and female separate, and while formerly they remained quietly in the dishes or aquaria, they now proceed to climb out, and show a tendency to wander over the building.

3. *Polar Bodies*.—I have seen these extruded in the egg of the tree frog. They are found at or near the apex of the black pole, and appear as two white spots with a black periphery. Sometimes they are quite near to each other. Again, I have seen them separated by quite a wide distance. They were extruded about one hour after the eggs were laid as nearly as could be calculated.

4. *Segmentation of the Eggs*.—The series of diagrams ordinarily found in text-books on embryology are exceedingly diagrammatic, and give an entirely erroneous impression as to the appearance of the segmenting egg, especially during the later stages. I found this to be the case in the eggs of the tree frogs (see above) and the common toad, and expected to find a parallel case in *Rana temporaria*,—that studied by Ecker, and from whom the text-book figures are taken. During the present spring ('91) I have procured the early stages of segmentation of this frog, and found it to agree in every particular with other species, and therefore to depart from the text-book or classical type. Rauber has given excellent figures of the later stages of the frog eggs, and in many points I have verified his account. The first furrow divides the egg into two equal halves. The second at right angles to this gives four equal segments. The third furrow is not equatorial, but lies nearest the dark pole of the egg, the result being in four equal black cells and four larger, but equal, light cells. At the next stage the marked regularity of the preceding stages is lost, and each of the eight cells divides, as it were, independently of the rest. The text-book figure at this sixteen-celled stage may be taken to represent an ideal to which the egg never attains. The division of the sixteen cells into thirty-two does not conform to any rule, although again, but in a less degree, Ecker's figures may be taken to represent in the most diagrammatic way possible the planes of cleavage. Without figures it is impossible to describe the precise method of segmentation; those of Rauber approximate, I believe, most nearly to the truth. In general, we may

say that up to the eight-celled stage the segmentation is very regular, but that after that no particular plane of division can be prophesied for any segment. Often during the sixteen-celled stage the upper eight (black) cells are arranged in almost a perfect bilateral symmetry, and not a radial one, as given by Ecker.

5. *Orientation of the Egg*.—The relation of the first plane of segmentation to the adult has attracted a great deal of interest during recent years. The relation found in the frog's egg has been already studied, with varying results. Newport's experiments in 1851, '53, '54, are, I think, the most to be relied upon, and during the present spring I have had the pleasure of verifying his results on a small scale. The eggs of the tree frog were used in the experiment. The outer layers of the jelly were removed from an egg which had not yet divided or had only undergone the first cleavage. A small, triangular piece of card-board was then cut out, and a drop of collodion placed on it. The egg with its thin layer of surrounding jelly was placed on the drop of collodion as soon as the latter began to stiffen, and card-board and egg were then immersed in a dish of water. With a pencil a line was drawn on the card-board corresponding to the plane of first division. The water was changed several times until all trace of ether was gone, and afterward set aside in a quiet and warm place. Several other eggs were prepared by the same process. At the end of forty-eight hours the medullary folds began to appear, and it was then seen that the plane between these corresponded exactly, in most cases, to the plane indicated on the card-board, and therefore the obvious conclusion is drawn that the first plane of division divides the egg into two parts, corresponding to the right and left halves of the adult body. In a few eggs the first plane was somewhat to the right or left of the mid-line of the adult. The embryo begins to rotate in the egg-capsule very soon after the appearance of the medullary folds, so that unless observations are made at the very first appearance of the folds the results will be falsified, on account of the rotation of the embryo from its original position. The eggs of the tree frogs are especially good for experiments such as these, on account of the rapidity with which they develop, decreasing therefore the possibilities of a secondary change in position of the egg after it has come to rest and its plane of division marked. I think it would be possible, by keeping the eggs in a warm room, to cause them to develop the medullary folds within twenty-four hours after the eggs are laid.

6. *Enclosure of the Light Pole by the Dark Pole*.—In studying a series of eggs from the segmentation period to the formation

of the blastopore, the so-called overgrowth or epibolic growth of the black cells has been observed. I am quite sure, however (except in the immediate region on the dorsal side of the blastopore, and later over its whole extent), that the yellow cells disappear from the surface not by an overgrowth of the first-formed black cells, *but by a process of splitting off of cells from the upper corner of the yellow cells themselves.* In other words, there is not a general migration of black cells, but each remains approximately in the position in which it was first formed, and new black cells are continually added at the periphery of the black cap by the splitting off of cells from the upper ends of the yellow cells, so that Balfour's sentence, that the disappearance of the yellow cells "is effected by the epiblast growing over the yolk at all points of its circumference," is somewhat misleading. As a corollary to what I have said, it follows, of course, that there is a continuous formation of new pigment taking place at the periphery of the black area *within the new cells that are being formed, and also within the ends of the yellow cells which go to form the new cells in this region.* I have not studied with sufficient care the gradual turning in of the cells around the rim of the blastopore. In one living egg, however, I saw in the dorsal region of the blastopore some of the cells forming the floor of the archenteron gradually disappear *within* the blastopore.

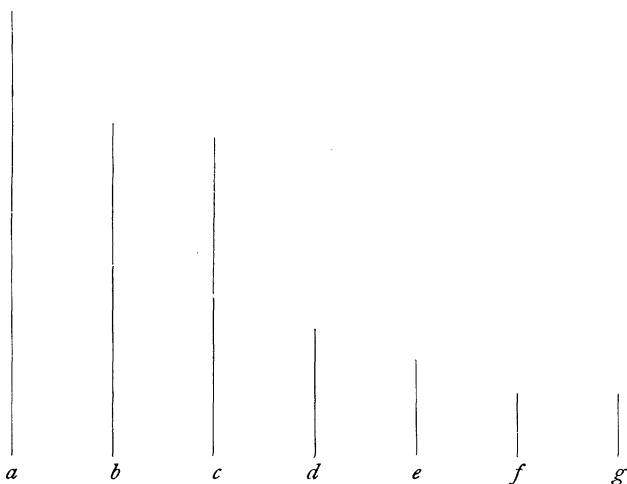
7. *Effect of Temperature.*—It is impossible to give any exact time to the different stages of development, as the time is directly proportional to the temperature of the water within certain limits. The highest temperature is not always the optimum, for several bunches placed in an incubator for hen's eggs were entirely destroyed. The freezing of the water in which the eggs are kept does not seem to injure the eggs in the least, but simply to retard their development. I have had eggs completely surrounded by ice, and afterward development quite normally. However, when the eggs themselves are actually frozen they seem to be destroyed, perhaps by the formation of ice spicules within them. The wood frogs, which lay their eggs so early, generally lose in this locality great numbers of them on account of getting caught in the ice. Those which are not so caught develop later, when the ice melts, and do not seem, in any way, to be injured by water at the freezing-point. I think there is here a most interesting field for experimentation by the physiological embryologist, and I regret I have not kept exact records of the effects of heat and cold.

8. *Times of Hatching, etc.*—The different species of frogs leave the jelly membrane at different ages. Some have the tail well developed, and are quite active. Others have the tail just appearing, and are

only able to twist their bodies slowly from side to side, as they cling to the jelly-mass by means of the suckers below the mouth.

The young tadpoles of the wood frog leave the water as small frogs in the late spring of the same year in which they were laid,—that is, become frogs in four to six months. Eggs collected about March 17th began to change to frogs about June 1st to 14th. These were kept in quite cool water, in a basement room, away from the sunlight.

At the time of transformation into tadpoles *a sudden decrease in the length of the intestine is brought about*. The tadpoles cease to eat, and the intestine is entirely freed from extraneous matter during this time. The change takes place at the same time that the tail is absorbed within the body (not dropped off, as popularly supposed), and at the same time the pair of fore feet, which were enclosed within the branchial fold, break through to the exterior. The intestines were removed and measured from the pyloric end of the stomach to the proximal end of the rectum. Their lengths are recorded in the accompanying table for the wood frog:



- (a) From a large tadpole, with whole tail and two large posterior feet. (b) From a tadpole, with whole tail and two large posterior feet. (c) From a tadpole, with whole tail and two large posterior feet. (d) From a young frog, tail beginning to disappear, and four feet. (e) From a young frog, $\frac{3}{4}$ tail and four feet. (f) From a young frog, $\frac{1}{2}$ tail and four feet. (g) From a young frog, no tail, and out of water two weeks.

9. *Methods of Technique*.—The eggs during the periods, in which it is difficult or impossible to remove the inner jelly membrane, can

be freed in the following manner: With a pair of sharp scissors each egg must be cut out from the general jelly-mass, retaining as small an amount of surrounding jelly as possible. It is then put into an alcoholic solution of picric acid for an hour or longer (one to twelve). The solution is prepared by saturating 35 per cent. alcohol with picric acid, and adding the same amount of sulphuric as in Kleinenberg's solution. The solution is not diluted, but used saturated with picric acid. The eggs are then washed for several hours in 35 per cent. alcohol, several hours in 50 per cent. alcohol, and placed in 70 per cent. for several days, changing the alcohol once or twice if necessary. About the second day the inner membrane begins to swell, due to a slow osmotic action, I think, as the membrane is stretched by tension from within. On the third or fourth day the swollen membrane may be pierced by a sharp needle, and the egg taken out, which is then placed permanently in 80 per cent. alcohol. The method is exceedingly simple, and consists largely in waiting a few days for the osmotic action to take place. Such eggs, if properly prepared, are in excellent histological condition. This simple method has proved so successful that I have not further experimented with it. It is possible that it may be improved by varying the strength of alcohol used, but I have not seen the need of looking further. The membrane does not swell in stronger alcohol than 70 per cent., and weaker would macerate the eggs.

Certain precautions are necessary in imbedding the eggs to prevent brittleness. This is obviated by soaking the eggs before imbedding, for several hours, in a solution of turpentine saturated with paraffine, and kept in a warm place,—not so hot as the water-bath (50° C.). Heat causes the egg to become brittle. This is obviated by the above process of soaking, so that the egg need not remain so long as an hour in the melted paraffine of the water-bath. In the younger stages there is no need for very thin sections, but sections 10 μ thick are sufficient for all purposes. If the sections are cut too thin the yolk tends to break up and crumble.—T. H. MORGAN, *May 1st, 1891.*